Advances in Extraction-Free Rapid Detection of Invasive Mussel Continuous-Flow Digital Droplet PCR in the Field

Preliminary Results from QZAP

Cody Youngbull and Shawn Devlin
Flathead Lake Biological Station
Polson, MT
cody.youngbull@flbs.umt.edu
shawn.devlin@flbs.umt.edu
406-982-3301

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FLATHEAD LAKE BIO STATION
UNIVERSITY OF MONTANA

Introducing the “DNA-Tracker”

Special thanks to:
Zane Lindstrom
Rod McNeil
David Sturgis
Dr. Andrew Hatch
Dr. Tathagata Ray

MBARI

GORDON AND BETTY MOORE FOUNDATION

SCCWRP Established 1969

U.S. FISH & WILDLIFE SERVICE DEPARTMENT OF THE INTERIOR

NSF

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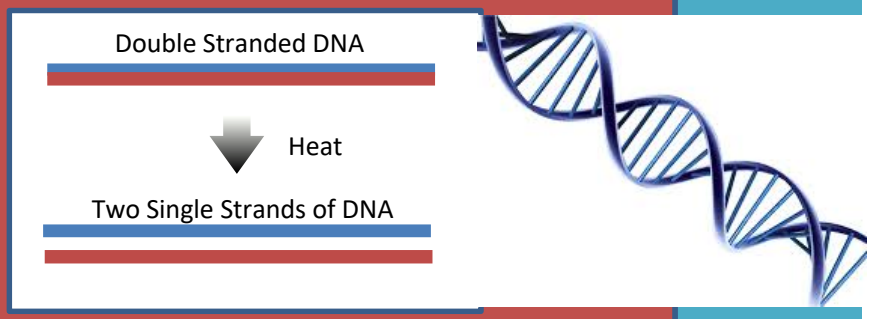
eDNA - Environmental DNA

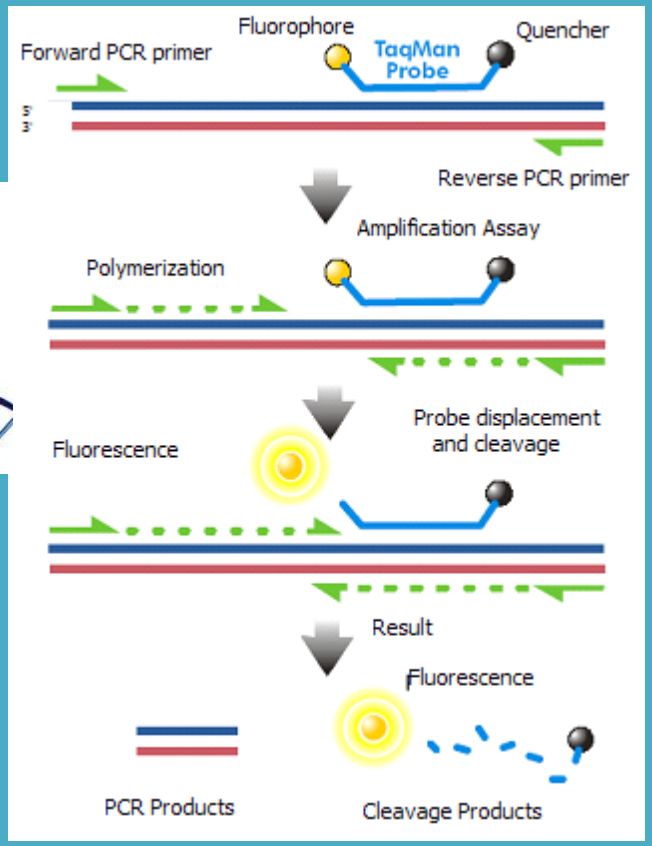
Challenges and Limitations

- Nucleic acid degradation
- Extraction efficiency variability
- Random spike events

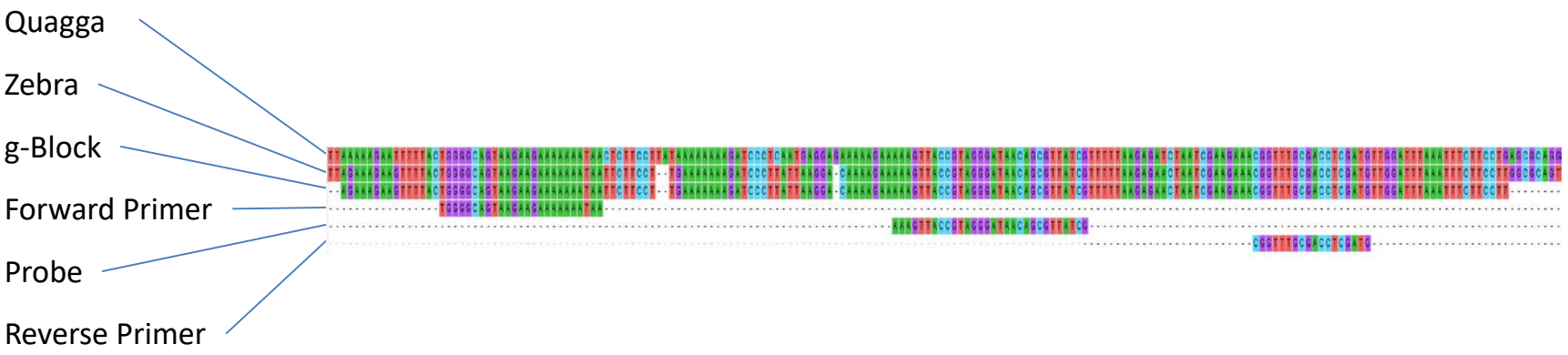
So how do we measure DNA?

Quantitative Polymerase Chain Reaction





DNA is used to detect eDNA.



1. Find

Nucleotide BLAST
nucleotide ▶ nucleotide

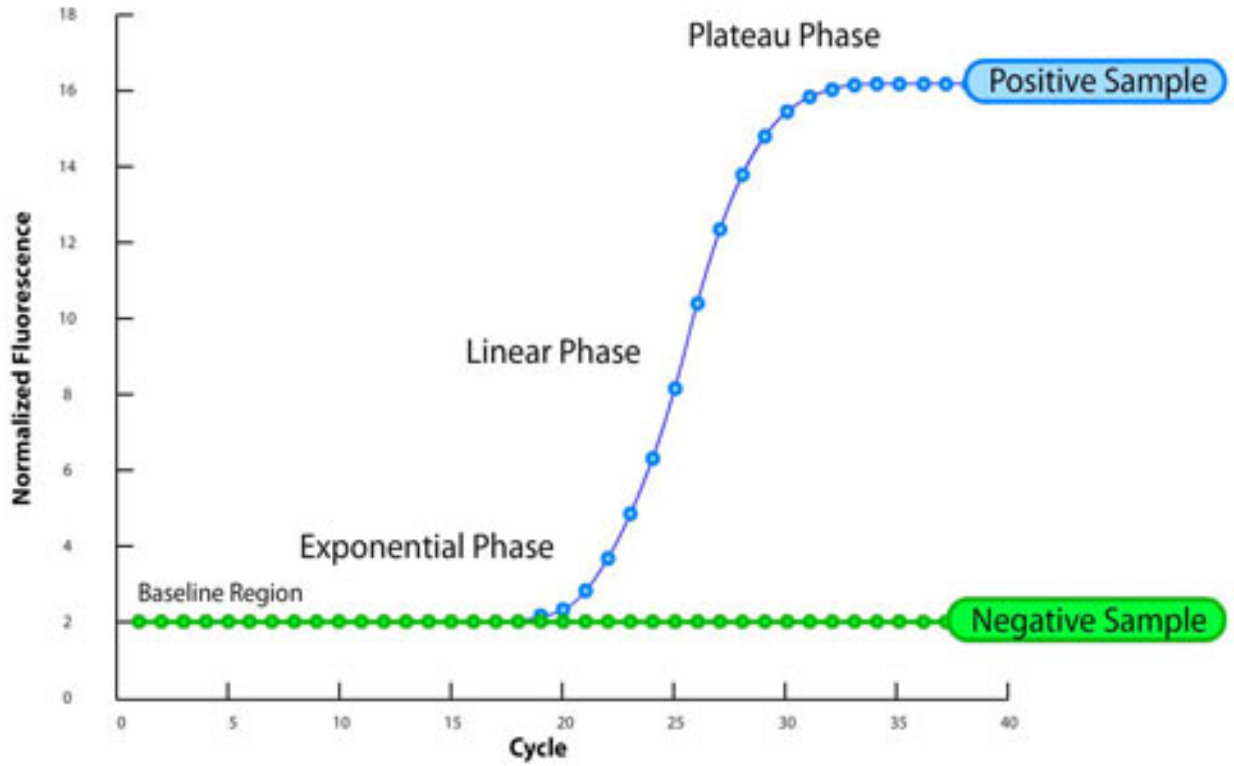
2. Design

MEGA Molecular Evolutionary Genetics Analysis

2. Order

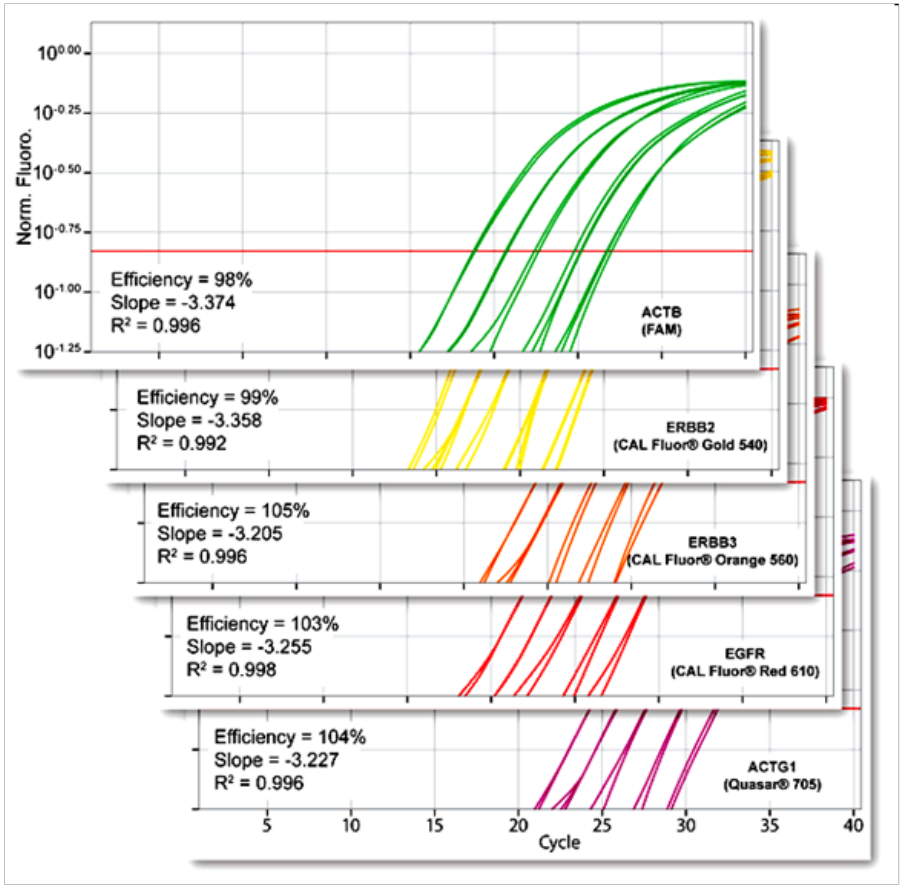
IDT
INTEGRATED DNA TECHNOLOGIES

Prototypical PCR data



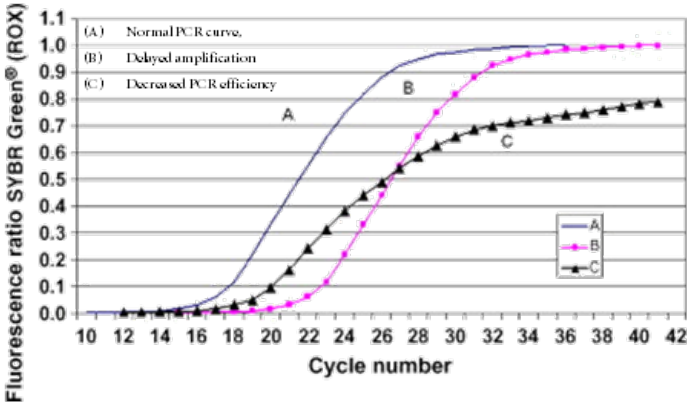
Real-Time qPCR requires a reference to interpret.

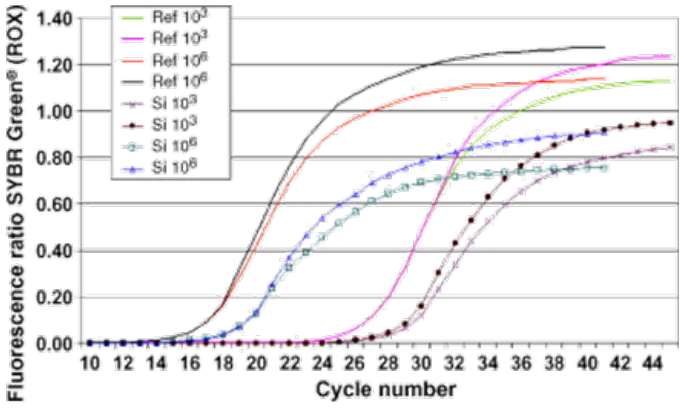
Quantify based on threshold



http://www.kapabiosystems.com/public/files/images/products/next-generation/kapa-probe/image2-modal.png

PCR can be troublesome





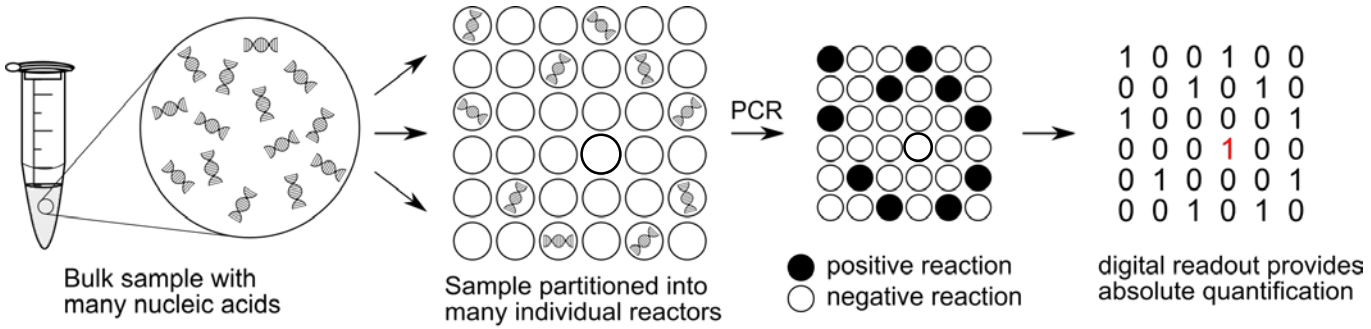
PCR Inhibition caused by interaction with silicon microfluidic surface material

Kolari et al. 2008, Real-time analysis of PCR inhibition on microfluidic materials

Principal of Operation – **Digital-Droplet qPCR**

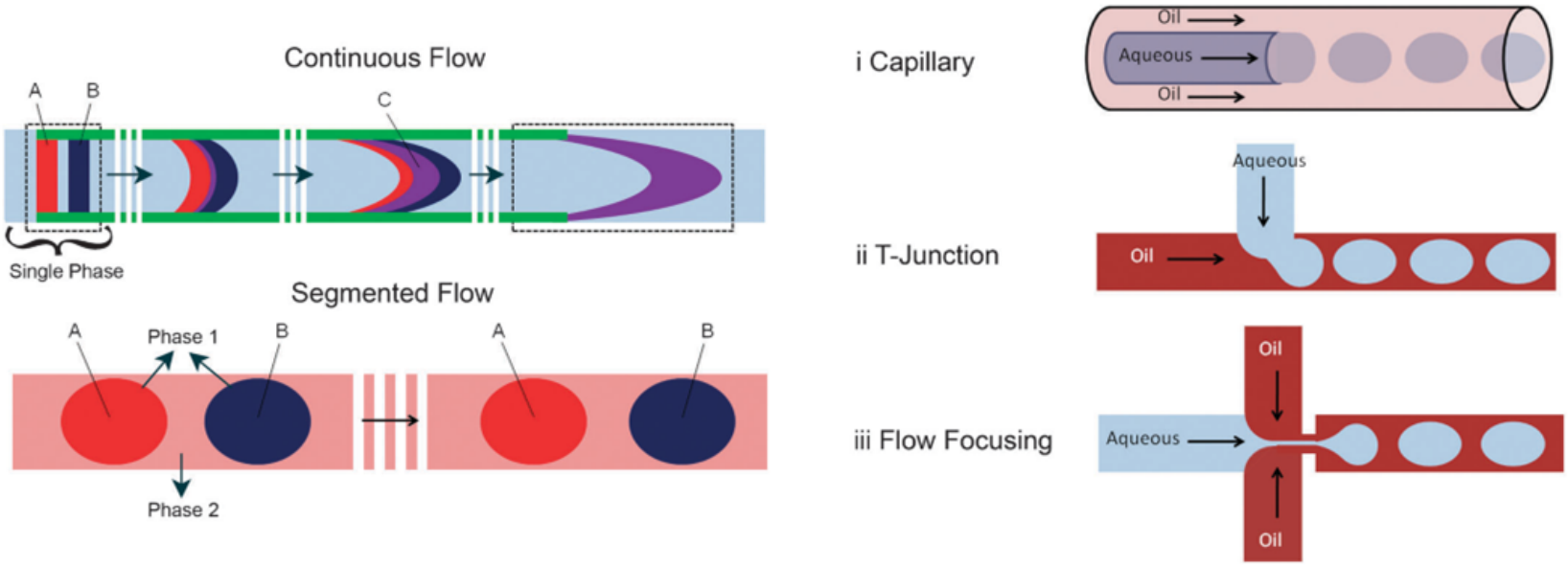
Capabilities Include:

- Single-molecule or single-cell detection Sensitivity
- Arbitrary Dynamic Range
- Arbitrary genome amplification
- Continuous qPCR quantification
- Absolute Titer Measurement: No control comparison needed



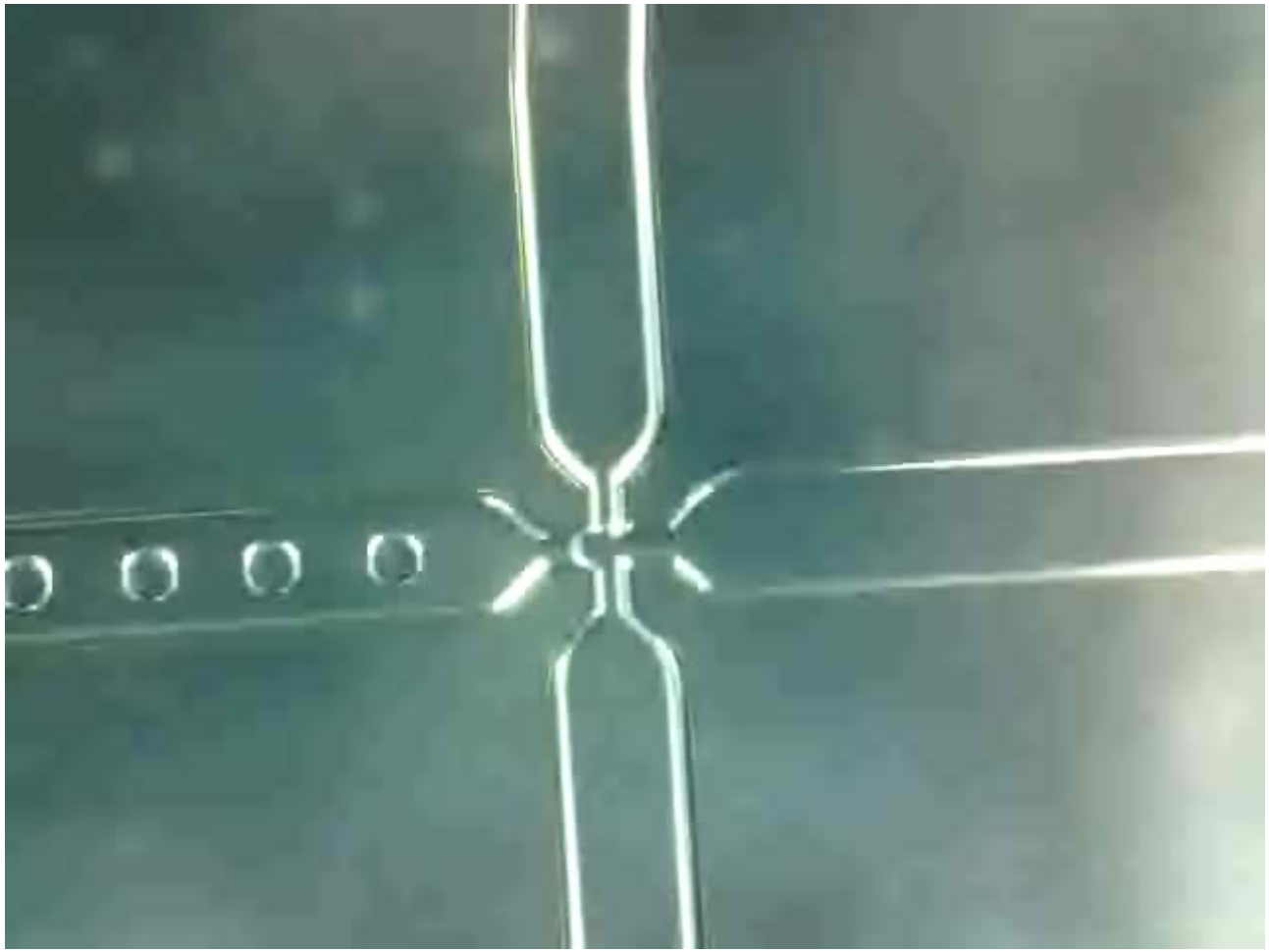
Emulsion Microfluidics enable digital droplet PCR

Minimize cross contamination, separate components

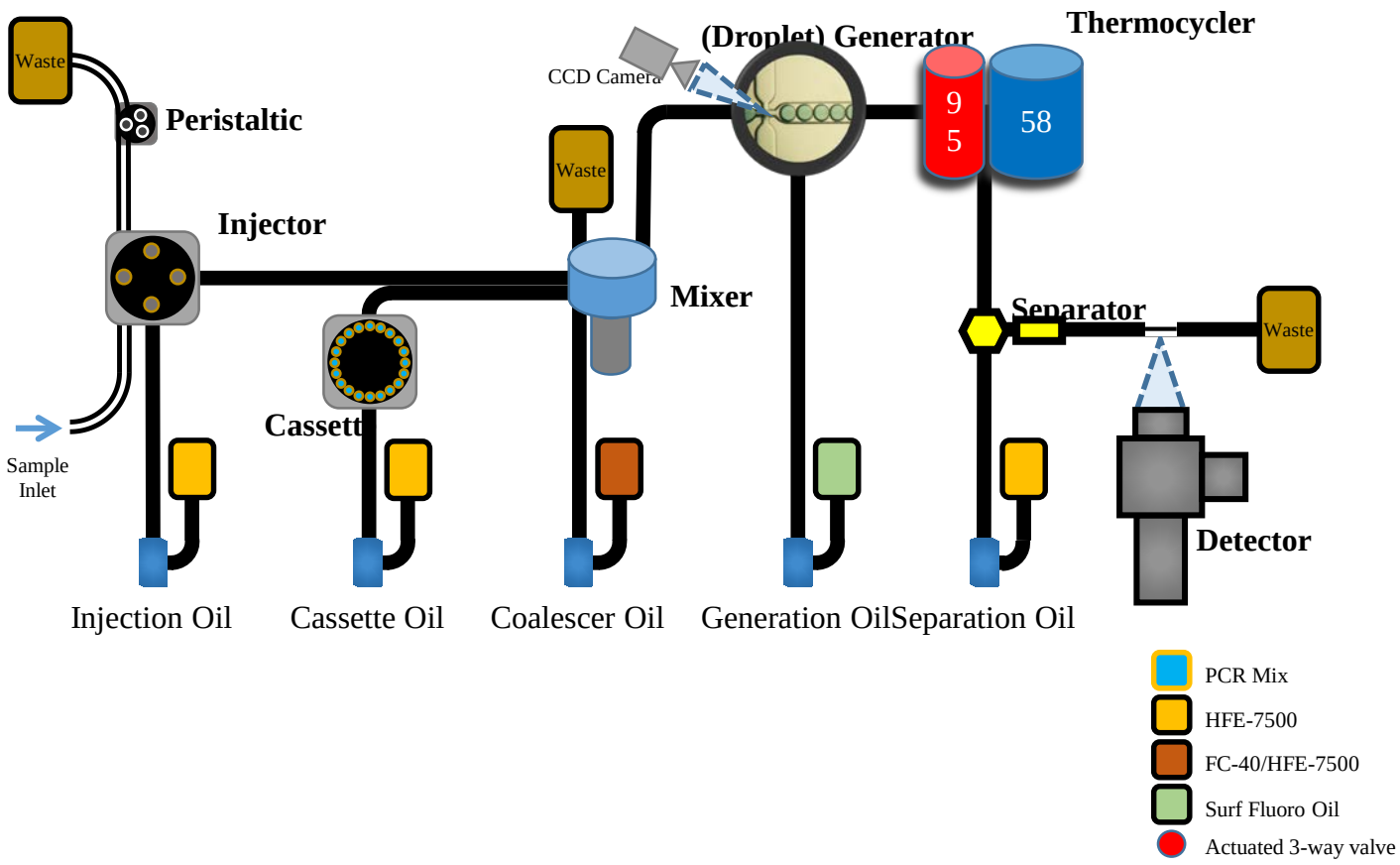


Independent reaction volumes with greater potential for sample purity, throughput, scalability, efficiency and accuracy

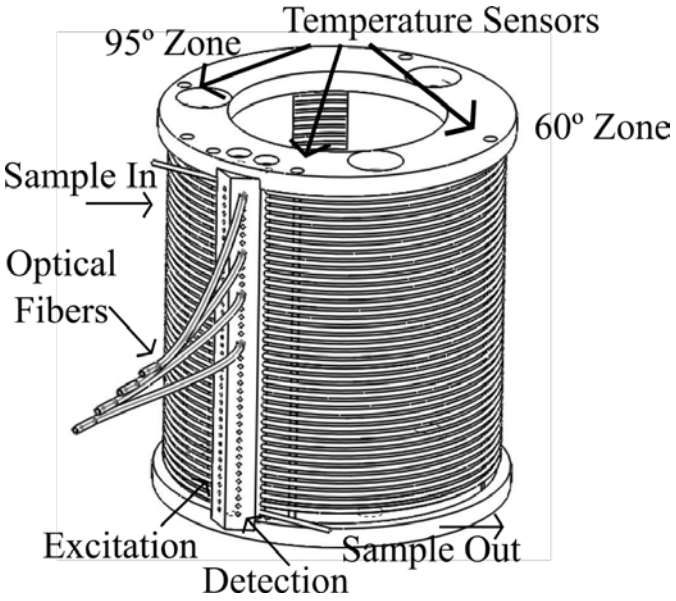
Slow Motion Droplet Formation

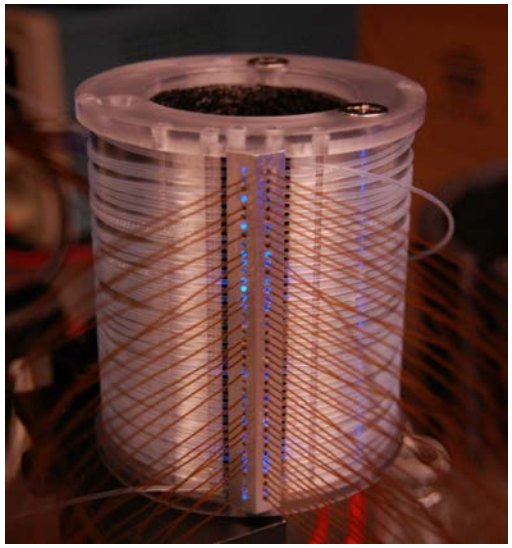


Fully Autonomous, Continuous-Flow ddPCR Instrument



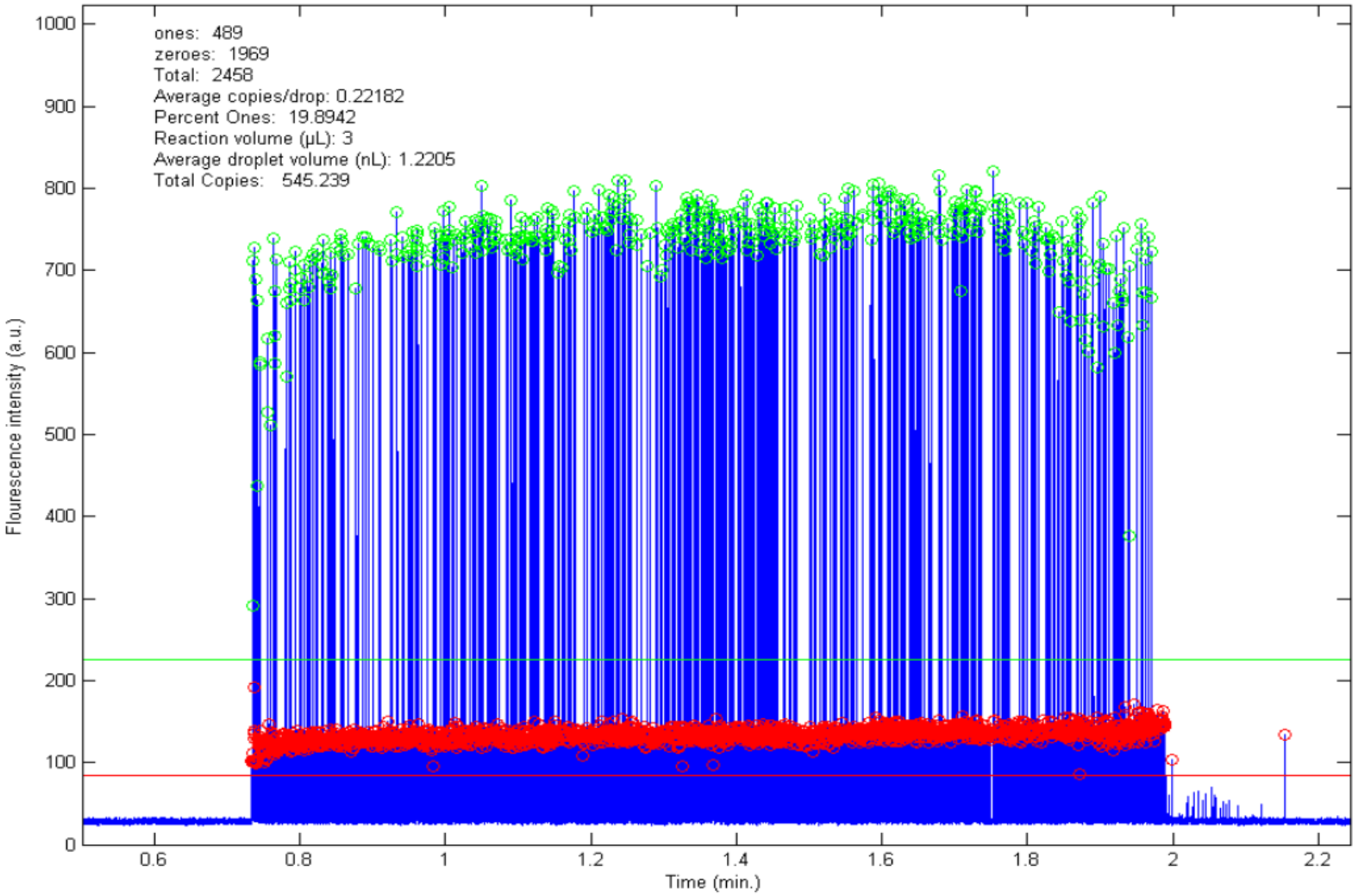
Low Power Thermal Cycling





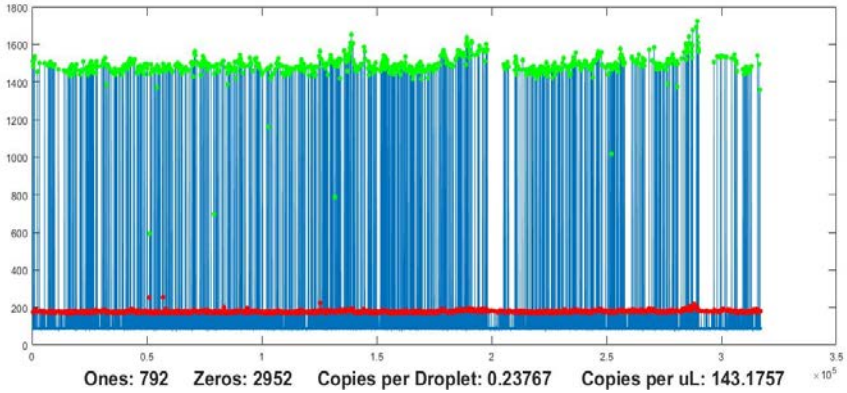
- Uses two zone cylindrical thermocycler with closed loop temperature feedback control
- 40 turns of FEP tubing wrapped around heater to achieve 40 PCR cycles
- Power consumption during PCR run <5W

Raw Data


ones: 489
zeroes: 1969
Total: 2458
Average copies/drop: 0.22182
Percent Ones: 19.8942
Reaction volume (µL): 3
Average droplet volume (nL): 1.2205
Total Copies: 545.239
Flourescence intensity (a.u.)
Time (min.)
0
100
200
300
400
500
600
700
800
900
1000
0.6
0.8
1
1.2
1.4
1.6
1.8
2
2.2

It’s not just about taking the lab to the field. It’s about meeting constraints and process flow.

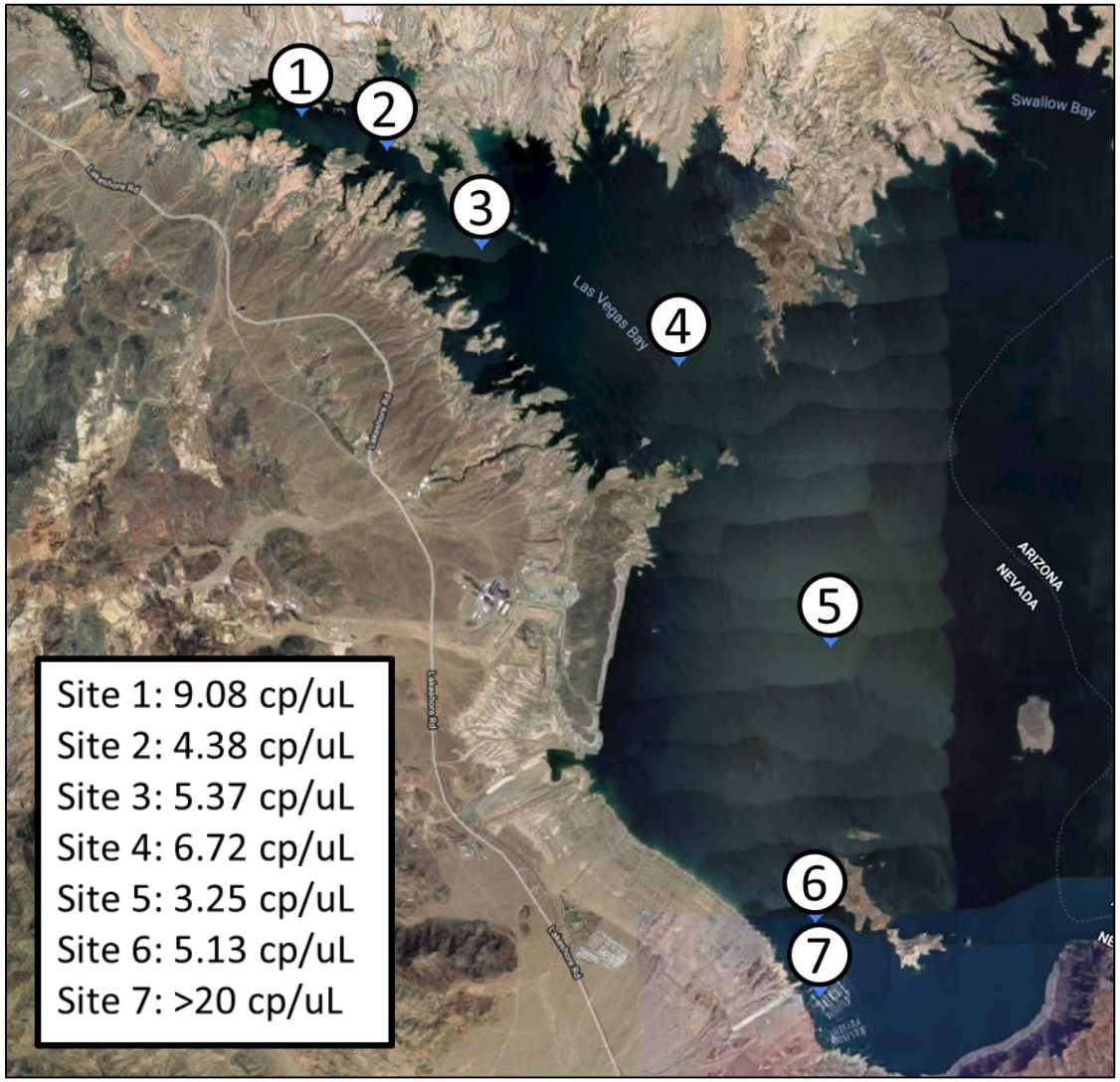
Quagga/Zebra eDNA Field-Ready Extraction Methods



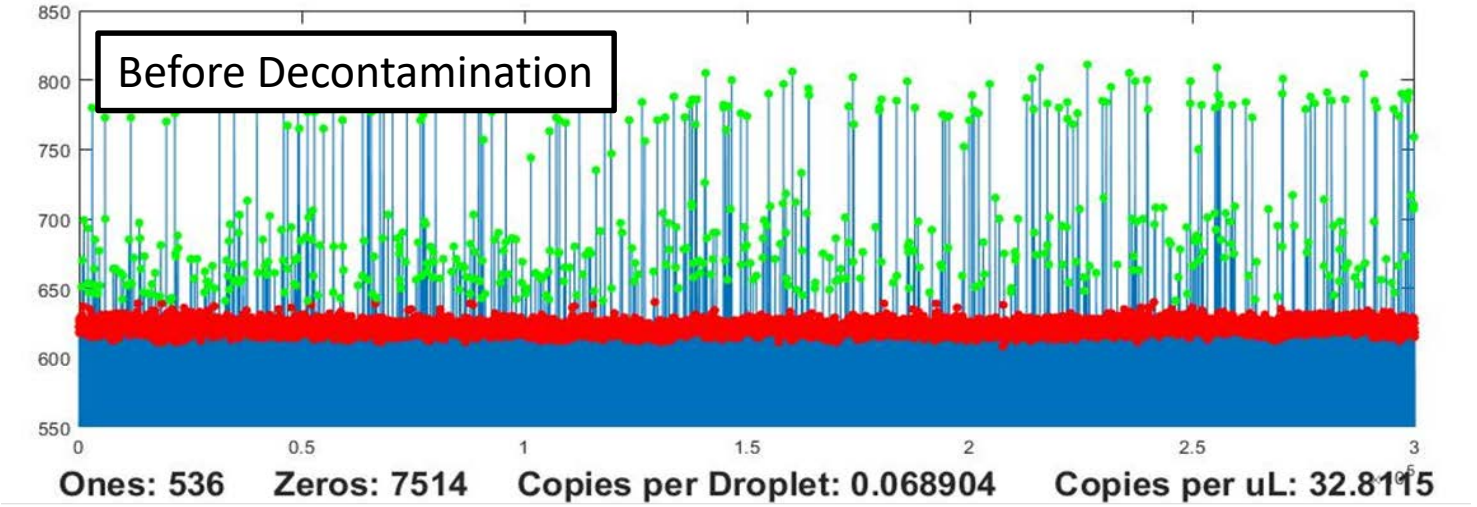
Practical Considerations of eDNA extraction

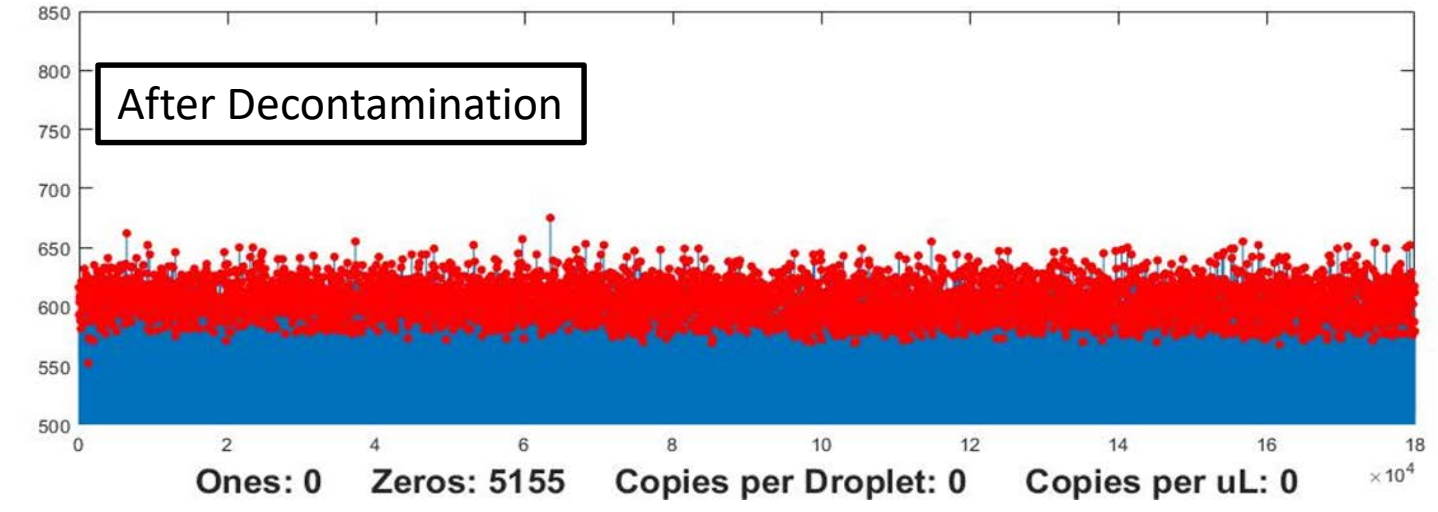
Criterion	Zygem	Zymo Micro prep	Master Pure	McNeil	Ultraclean	QuigenWater
cost per test	$3.00	$3.62	$2.17	$0.30	$3.20	$2.75
extraction efficiency	4.55ng/ul	1.26ng/ul	4.47ng/ul	0.34ng/ul	5.08ng/ul	3.44ng/ul
Temperature requirements						
4 C					10 min	10 min
37C		5 min incubate	30 incubate			
55C		10 min				
65C			15min	5min	10min	10 min
75C	5min					
95C	2min		5min	2min		
centrifugation	no	yes 3x	yes 2X	no	yes 1X	yes 2X
vortex mixing	yes 2X	yes 2X	yes 3X	yes 2x	yes 5x	hor vortex yes 3x
total procedural time	20 min	50 min	85 min	15 min	70 min	50 min
reagents	3	5	4	2	5	4
specialty tubes	no	yes	yes	no	yes	yes
reagent refridg	no	no	no	no	no	no

Free-DNA Gradient in Lake Mead



On-Site Validation of Decontamination





Watercraft Inspection Stations

DNA Tracker as an inspection tool

Will the DNA Tracker
Be Integrative or Interruptive
Timeframes:

- **Sampling**
- **Results**
- **Communication**

Public Perception

Truly Early Detection

Near Real-time Detection

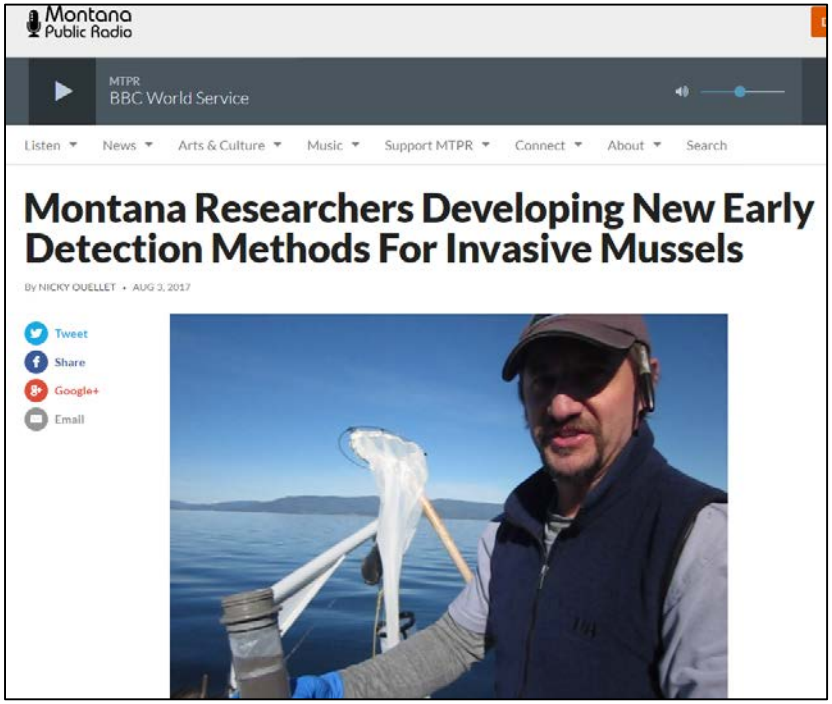
…if detected early and removed before extensive reproduction, it is possible to prevent a successful invasion of zebra mussels"

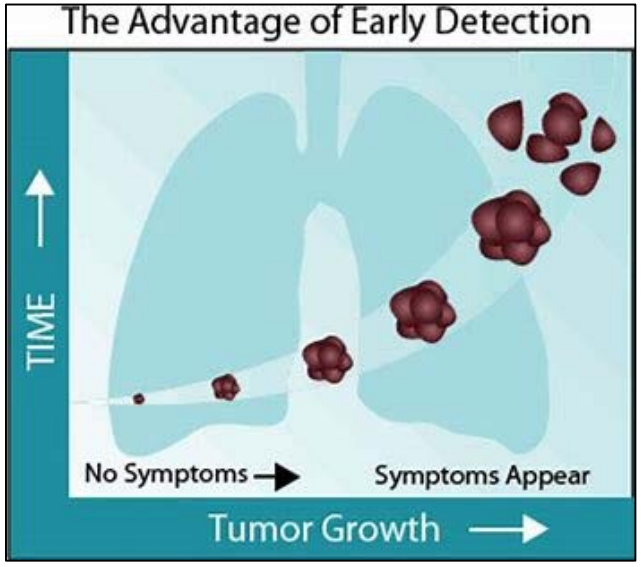
Wimbush et al 2009

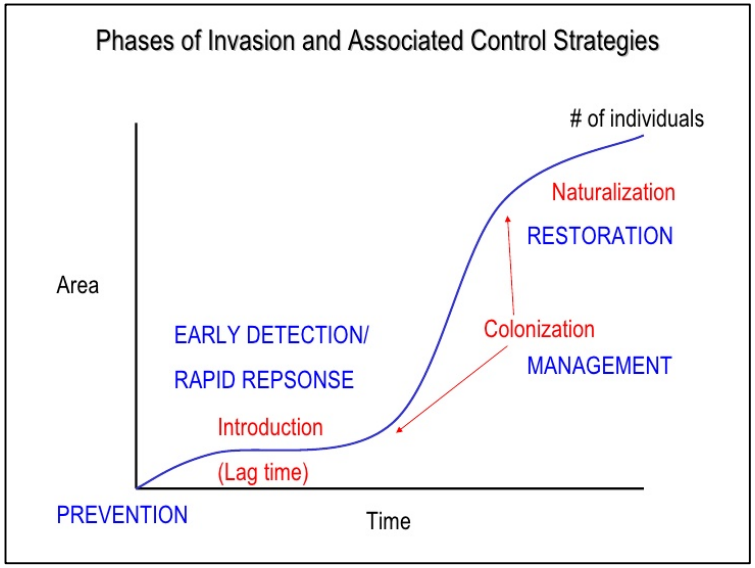
How Early is Early Enough?

Early Detection Research Needs

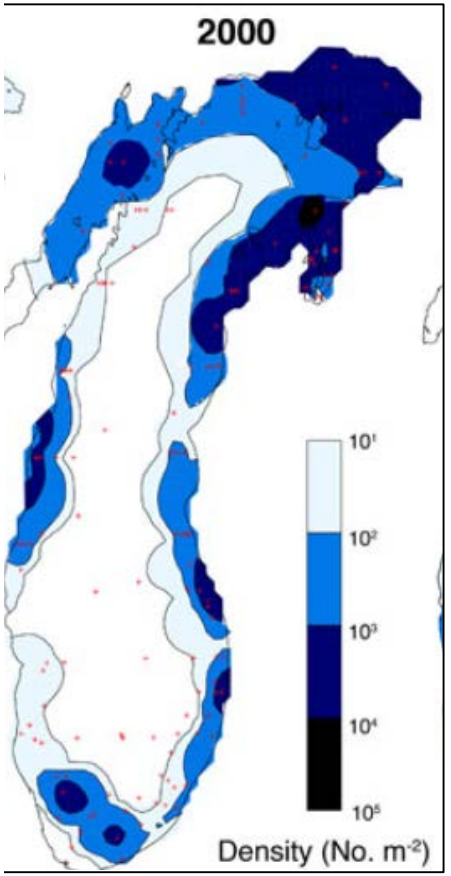
- Correlate copies/µL and Potential Density
- Determine lower limits of sampling detection
- Raw Filtered water vs. Grab Sample, Tow Sample







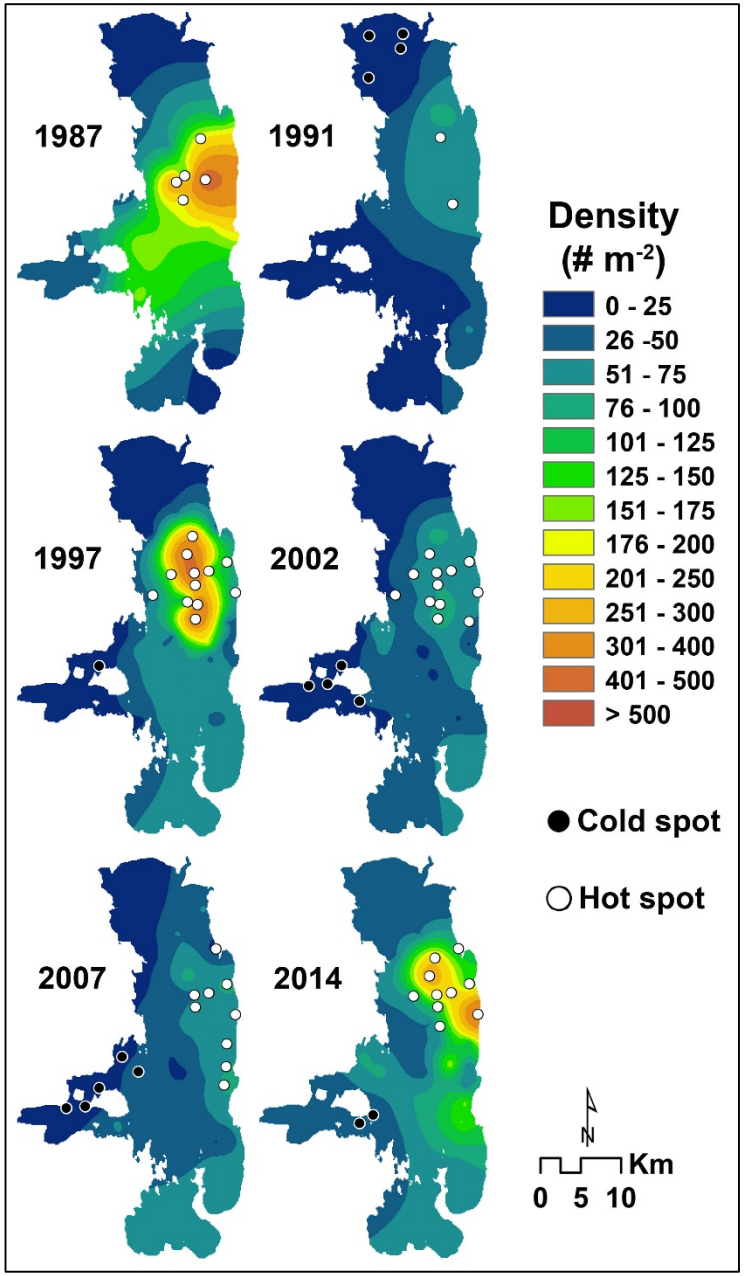
Plume or Sou Tracking



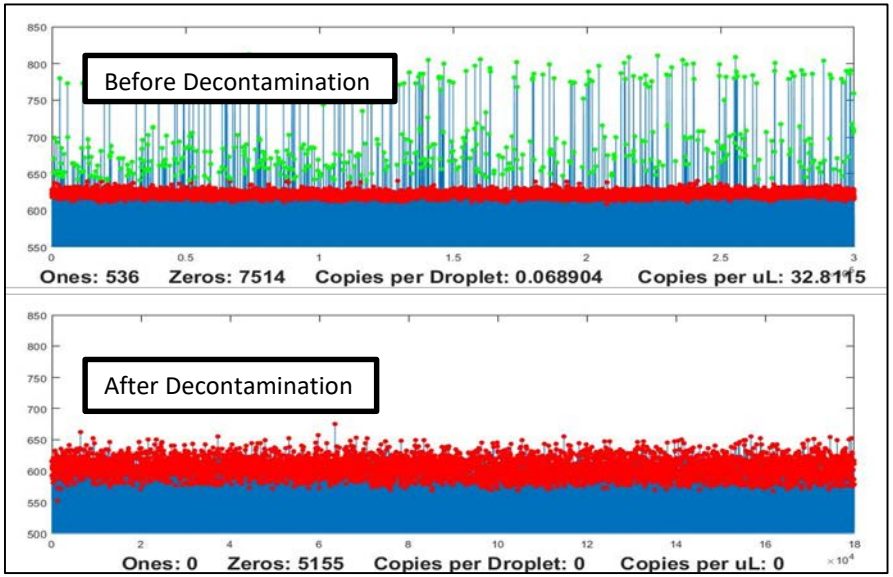
Positive Detection
NOW WHAT?

Source Tracking Research Needs

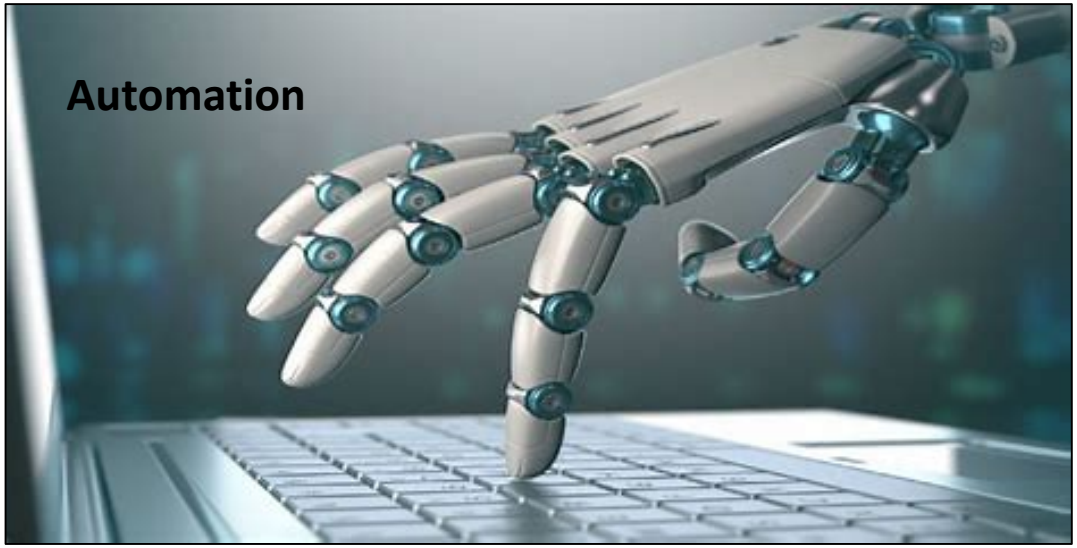
- Develop best Search Patterns/Survey Techniques
- Spatial Interpolation Approaches
- Real time mapping software applets
- Role of Currents and weather

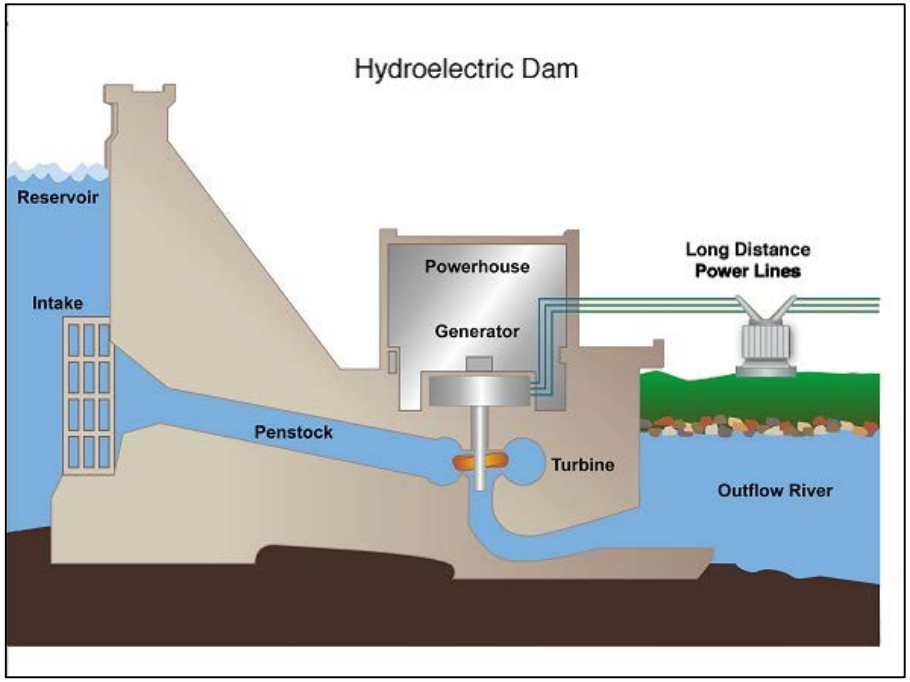


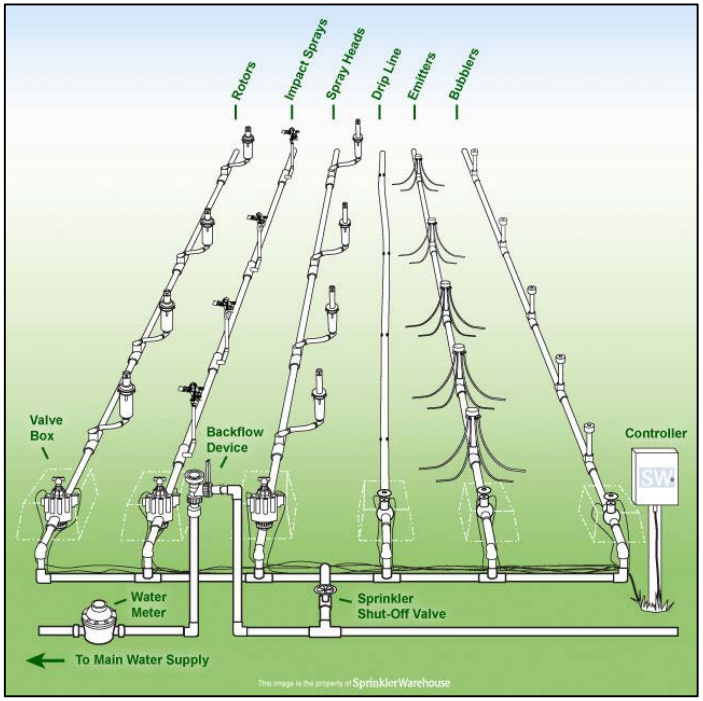
Decontamination Assurance

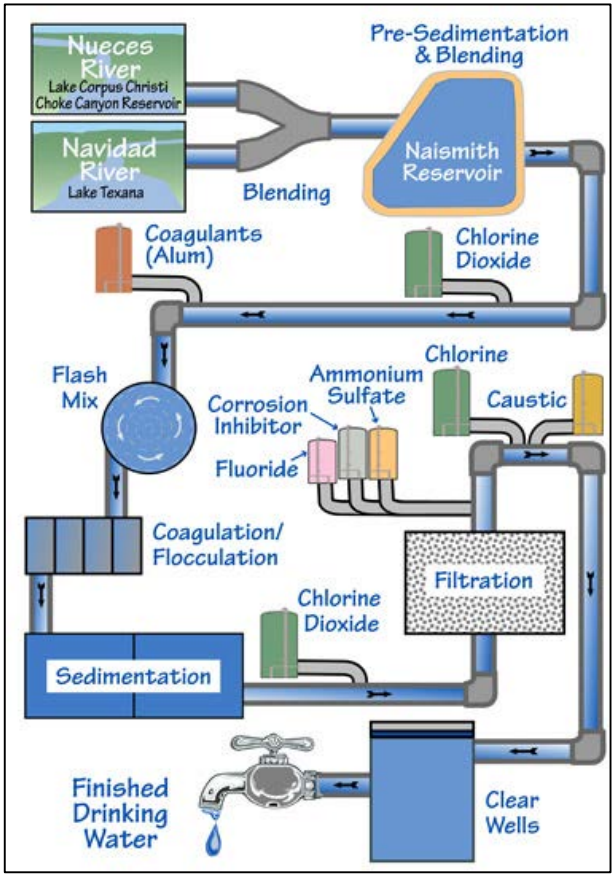


Near Future Developments




Hydroelectric Dam
Reservoir
Intake
Powerhouse
Generator
Long Distance
Power Lines
Penstock
Turbine
Outflow River


Rotors
Impact Sprays
Spray Heads
Drip Line
Emitters
Bubblers
Valve Box
Backflow Device
Controller
Water Meter
Sprinkler Shut-Off Valve
To Main Water Supply


Nueces River
Lake Corpus Christi
Choke Canyon Reservoir
Navidad River
Lake Texana
Pre-Sedimentation & Blending
Blending
Naismith Reservoir
Coagulants (Alum)
Chlorine Dioxide
Flash Mix
Chlorine
Ammonium Sulfate
Corrosion Inhibitor
Caustic
Fluoride
Coagulation/ Flocculation
Filtration
Chlorine Dioxide
Sedimentation
Finished Drinking Water
Clear Wells

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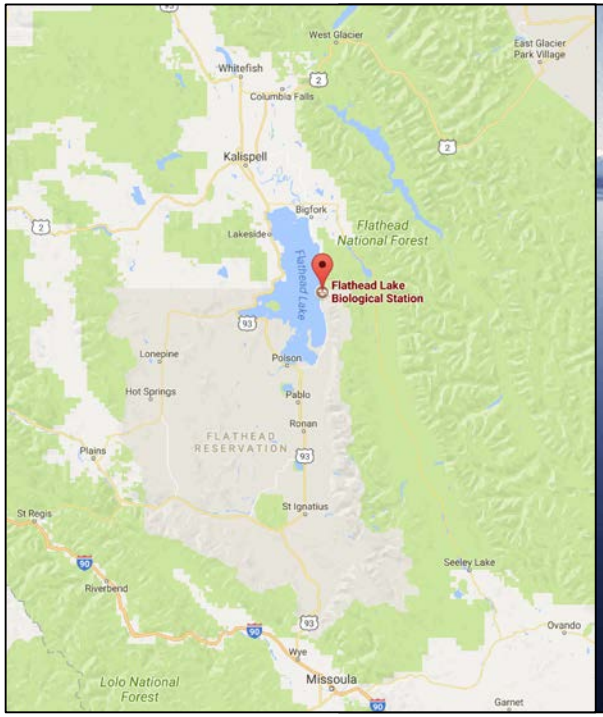
Looking for Questions, Applications, and Partners.



cody.youngbull@flbs.umt.edu
shawn.devlin@flbs.umt.edu
406-982-3301